

490

POTENTIAL MECHANISM OF ALENDRONATE INHIBITION UPON OSTEOPHYTE FORMATION IN THE RAT MODEL OF POST-TRAUMATIC OSTEOARTHRITIS: AN IN-VIVO MICRO-CT, ELECTRON PROBE AND HISTOLOGIC STUDY

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Purpose: Our study focused on the reported ability of the bisphosphonate drug Alendronate (ALN) to block osteophytosis in rat models of post-traumatic osteoarthritis (PTOA), a process that the closely related Risedronate has not been shown to influence. Our purpose was to better elucidate the mechanism whereby ALN is seen to inhibit osteophyte (OST) formation and slow disease progression in rat surgical models of PTOA.

Methods: PTOA pathogenesis was induced in 12 rats by medial meniscectomy (MMx) surgery. We utilized *in-vivo* microfocal computed tomography (Micro-CT) to measure temporal bony adaptations at 4 and 8 weeks after surgery in those rats with or without ALN treatment, and compared them to six sham-operated control rats. A pulse of elemental Strontium (Sr) was administered by oral gavage in the last 10 days to map bone turnover in developing PTOA using Electron Probe Micro Analysis (EPMA). Histologic studies were then undertaken on safranin-O/fast green and tetrachrome stained decalcified sections to examine articular cartilage health and OST formation.

Results: Micro-CT volumetric measurements of OST (Figure 1a) revealed that ALN treatment inhibited OST development by 40% and 51% at 4 and 8 weeks after surgery, respectively. OST in ALN group were measured with reduced BMD values (i.e., more cartilaginous in composition) compared to the untreated MMx group. Histological examination confirmed the OST inhibitory effect of ALN, and provided evidence of reduced articular cartilage degradation, compared to untreated MMx rats. EPMA mapped uniform deposition of Sr tracer over actively remodeling trabecular surfaces in normal control rats. That pattern, however, was altered after MMx resulting in greater Sr signal localized to developing OST margins (Figure 1b). ALN treatment reduced the incorporation of Sr tracer in OST margins, confirming the reduced mineralization of tissues in developing OST.

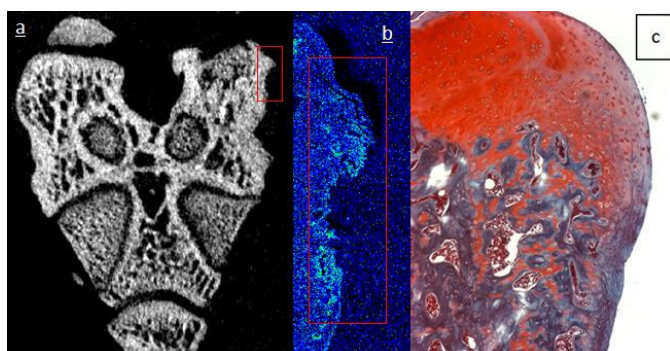


Fig. 1. Bony osteophyte, detected using: (a) Micro-CT, (b) Sr EPMA, (c) Histology.

Conclusions: Our study confirmed that ALN administration will reduce OST formation in a rat model of PTOA, partially through the inhibition of secondary remodeling of OST. ALN preserved articular cartilage health by inhibiting secondary bone turnover and/or sclerosis of periarticular and subchondral bone. Our results support the indication of ALN in the prevention of OST formation, which if administered immediately after traumatic injury (over a transient therapeutic window), may prove an effective disease-modifying drug treatment to slow the progression of secondary OA.

491

SUSTAINED STIMULATION OF CARTILAGE BIOSYNTHETIC ACTIVITY AFTER INTRA-ARTICULAR INJECTION OF AN ENGINEERED IGF-1

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Purpose: IGF-1 stimulates cartilage repair, but is not a practical arthritis therapy due to its short half-life and systemic side-effects. Fusing

IGF-1 with a heparin-binding domain generates an IGF-1 (termed "HB-IGF-1") that sticks to cartilage through binding to its abundant chondroitin sulfated proteoglycans. In the present study we tested whether intra-articular injection of HB-IGF-1 provides long-term delivery and stimulation of cartilage biosynthetic activity *in vivo*.

Methods: Proteins: HB-IGF-1 was created by N-terminal fusion of a modified heparin-binding domain from human HB-EGF with human IGF-1. The protein was purified after expression in *E. coli*. Recombinant human IGF-1 (Increlex) was from Ipsen. *Rat studies:* 3-month-old Lewis rats received intra-articular injection of 100 microliters saline containing 100 micrograms HB-IGF-1, IGF-1, or no additions. *Ex vivo proteoglycan biosynthesis:* The menisci of the injected joint were cleaned of all attached connective tissues and then incubated in serum-free medium with 35S-sulfate for 18 hours. After washing in cold medium and digesting the cartilage, radiolabel incorporation was measured by liquid scintillation.

Results: After intra-articular injection, unmodified IGF-1 was not detectable in extracts of harvested cartilage by Western analysis. In contrast, HB-IGF-1 remained detectable in articular and meniscal cartilages for 6–8 days after injection. Two days after injection, unmodified IGF-1 had no effect on meniscal cartilage proteoglycan synthesis, whereas HB-IGF-1 caused a 2.1-fold stimulation of proteoglycan synthesis relative to saline only ($N = 5$ rats/group, $P < 0.05$ by t-test; Figure 1). Four days after injection, there remained a significant stimulation by HB-IGF-1 (1.8-fold, $P < 0.05$), but not IGF-1, relative to saline.

Conclusions: These data demonstrate that a heparin-binding IGF-1 fusion protein provides sustained delivery and functional effect *in vivo* after intra-articular injection in rats. Heparin-binding fusions may represent a new strategy for sustained local delivery of therapeutic proteins to cartilage.

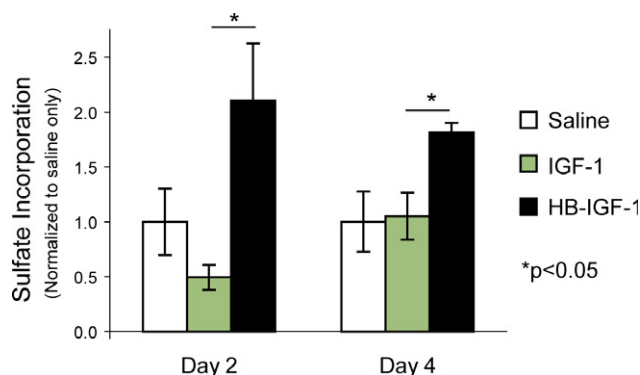


Fig. 1.

492

SELECTIVE INHIBITION OF INDUCIBLE NITRIC OXIDE SYNTHASE PREVENTS LIPID PEROXIDATION IN CARTILAGE FROM PATIENTS WITH OSTEOARTHRITIS

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Purpose: Emerging evidence indicates that nitric oxide (NO), which is increased in osteoarthritic (OA) cartilage, plays a role in 4-hydroxynonenal (HNE) generation through peroxynitrite formation. HNE is considered as the most reactive product of lipid peroxidation (LPO). We have previously reported that HNE levels in synovial fluids are more elevated in knees of OA patients compared to healthy. We also demonstrated that HNE induces a panoply of inflammatory and catabolic mediators known for their implication in OA cartilage degradation. The aim of the present study was to investigate the ability of inducible NO synthase (iNOS) inhibitor, L-NIL, to prevent HNE generation through NO inhibition in human OA chondrocytes.

Methods: Cells and cartilage explants were treated with or without either an NO generator (SIN or interleukin 1beta (IL-1β)) or HNE in absence or presence of L-NIL. Protein expression of both iNOS and free-radical-generating NOX subunit p47 (phox) were done by Western blot. iNOS mRNA detection was measured by real-time RT-PCR. HNE production was analysed by ELISA, Western blot and immunohistochemistry. S-nitrosylated proteins were evaluated by